SSR Journal of Medical Sciences (SSRJMS)

Homepage: https://ssrpublisher.com/ssrjms/

Volume 3, Issue 3, 2026 ISSN: 3049-0375

Comparative Neuroprotective Effects of *Newbouldia Laevis Leaf* Extract and Tocopherol on Aluminium Chloride–Induced Cerebellar Cortical Damage in Wistar Rats

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Received: *11.02.2026* | ***Accepted:*** *27.02.2026* | ***Published:*** *01.03.2026*

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DOI: *10.5281/zenodo.18819424*

Abstract **Review Article**

Aluminium chloride ($AlCl_3$) induces neurotoxicity primarily through oxidative stress, leading to cerebellar damage and motor dysfunction. This study evaluated the comparative neuroprotective effects of ethanolic leaf extract of Newbouldia laevis (NLE) and tocopherol against $AlCl_3$-induced cerebellar cortical damage in Wistar rats. Forty-eight male Wistar rats were divided into six groups (n=8): control, $AlCl_3$ (100 mg/kg/day, oral, 28 days), $AlCl_3$ + NLE (200 mg/kg), $AlCl_3$ + NLE (400 mg/kg), $AlCl_3$ + tocopherol (100 mg/kg), and $AlCl_3$ + NLE (200 mg/kg) + tocopherol (100 mg/kg). Behavioral assessments (open field and rotarod tests), biochemical analyses (MDA, SOD, CAT, GSH), histological examination (H&E staining) and immunohistochemistry (GFAP and caspase-3) were performed. $AlCl_3$ significantly increased oxidative stress markers, impaired motor coordination, induced Purkinje cell loss, astrogliosis, and apoptosis. NLE and tocopherol treatments attenuated these effects, with dose-dependent improvements observed for NLE and synergistic benefits in the combination group. These findings suggest that N. laevis leaf extract offers comparable or superior neuroprotection to tocopherol, potentially through multifaceted antioxidant and anti-apoptotic mechanisms.

Abbreviations: $AlCl_3$, aluminium chloride; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde; NLE, Newbouldia laevis ethanolic leaf extract; SOD, superoxide dismutase.

OBJECTIVE: To investigate the neuroprotective properties of Newbouldia laevis and Tocopherol in $AlCl_3$-Induced Cerebellar Damage

Keywords: Newbouldia laevis, Tocopherol, Aluminium chloride, Cerebellar cortex, Neuroprotection, Oxidative stress, Wistar rats.

1. INTRODUCTION

Aluminium (Al) is a ubiquitous environmental metal with no known physiological function in humans. Chronic exposure to Aluminium and its compounds has been strongly associated with neurodegenerative processes, including cognitive impairment, motor dysfunction and neuropathological changes reminiscent of Alzheimer's disease and other neurodegenerative disorders (Exley, 2013; Kumar & Gill, 2014; Ribes et al., 2021). Aluminium chloride ($AlCl_3$) is commonly employed in experimental models due to its ability to cross the blood–brain barrier and induce oxidative stress, mitochondrial dysfunction, neuroinflammation and neuronal apoptosis (Kumar et al., 2009; Singla & Dhawan, 2020).

The cerebellum plays a crucial role in motor coordination, posture and balance, and its Purkinje neurons are highly susceptible to oxidative and excitotoxic insults. Aluminium exposure has been shown to cause Purkinje cell loss, astrogliosis, vacuolation of the molecular layer, and impaired motor performance in rodents (Nedzvetsky et al., 2006; Rajendran et al., 2023). Oxidative stress remains a central mechanism of aluminium neurotoxicity, characterized by excessive reactive oxygen species (ROS) generation, lipid peroxidation, and depletion of endogenous antioxidant defenses (Kumar & Gill, 2014; Majumdar et al., 2014).

Natural products with antioxidant and anti-inflammatory properties have gained attention as potential neuroprotective agents against aluminium-induced neurotoxicity (Ogundipe et al., 2024). Newbouldia laevis (Bignoniaceae), commonly known as the "boundary tree," is widely used in Nigerian ethnomedicine for the management of neurological, inflammatory, and metabolic disorders (Eyong et al., 2013; Usman et al., 2015). Phytochemical analyses reveal that N. laevis leaves are rich in flavonoids such as apigenin and quercetin, as well as phenolic compounds with potent antioxidant, anxiolytic, anticonvulsant, and neuroprotective activities (Azubuike et al., 2019; Azubuike et al., 2021; Oloyede et al., 2017).

Tocopherol (vitamin E), a lipid-soluble antioxidant, protects neuronal membranes from oxidative damage by scavenging free radicals and inhibiting lipid peroxidation. Several studies have demonstrated its protective role against aluminium-induced neurotoxicity in different brain regions (Nedzvetsky et al., 2006; Albrahim et al., 2023; Sharma et al., 2024). However, direct comparative studies evaluating plant-based antioxidants and vitamin E, particularly in the cerebellum, remain limited.

Therefore, this study aimed to comparatively assess the neuroprotective efficacy of ethanolic leaf extract of Newbouldia laevis and tocopherol, alone and in combination, against $AlCl_3$-induced cerebellar cortical damage in Wistar rats.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

Fresh leaves of Newbouldia laevis were collected from Ihiagwa, Imo, State Nigeria, and authenticated by a plant taxonomist, with a voucher specimen deposited in the University Herbarium. The leaves were air-dried, blended and extracted by cold maceration using 70% ethanol. The extract was filtered, concentrated, and dried to yield 15.2% w/w.

2.2. Animal and experimental design

Forty- five adult male Wistar rats (150–200 g) were used. Animals were housed under standard laboratory conditions with free access to food and water. Rats were randomly assigned to five experimental groups as previously described. $AlCl_3$ was administered orally at 100 mg/kg/day for 28 days, a dose shown to reliably induce neurotoxicity (Akinyemi et al., 2015; Singhal et al., 2015). All experimental procedures complied with institutional ethical guidelines.

Group 1: Control (distilled water)

Group 2: $AlCl_3$ (100 mg/kg/day, oral)

Group 3: $AlCl_3$ + NLE (200 mg/kg)

Group 4: $AlCl_3$ + NLE (400 mg/kg)

Group 5: $AlCl_3$ + tocopherol (100 mg/kg)

2.3. Behavioral assessments

Motor activity was evaluated using the open field test (line crossings, rearing, grooming) and rotarod test (latency to fall) on day 21. These paradigms are sensitive indicators of cerebellar dysfunction and aluminium-induced motor deficits (Godam et al., 2024).

2.4. Biochemical analysis

Cerebellar tissues were homogenized and analyzed for oxidative stress markers. MDA levels were quantified as an index of lipid peroxidation, while SOD, CAT and GSH activities reflected endogenous antioxidant status (Majumdar et al., 2014; Oliveira et al., 2018).

2.5. Histopathology and immunohistochemistry

Cerebella were fixed in 10% formalin, paraffin-embedded, sectioned (5 μm), and stained with H&E for morphology examination. Immunohistochemistry was performed for GFAP (astrogliosis) and cleaved caspase-3 (apoptosis) using standard protocols with semi-quantitative scoring. (Ribes et al., 2021; Rajendran et al., 2023).

2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used ($p < 0.05$ significant).

3. RESULTS

3.1. Behavioral findings

$AlCl_3$-treated rats exhibited significant reductions in locomotor activity and motor coordination, consistent with cerebellar dysfunction (Nedzvetsky et al., 2006). NLE treatment produced dose-dependent improvements, with the 400 mg/kg dose and combination therapy resoring performance to near-control levels.

Table 1. Effects on open field test and rotarod performance

Group	*Control*	*$AlCl_3$*	*$AlCl_3$ + NLE 200 (mg)*	*$AlCl_3$ + NLE 400(mg)*	*$AlCl_3$ + Vit E*	*$AlCl_3$ + NLE 200 + Vit E*
Line Crossings	85 ± 7	45 ± 5*	65 ± 6#	78 ± 6#	70 ± 5#	82 ± 7#
Rearing	28 ± 3	12 ± 2*	18 ± 2#	25 ± 3#	20 ± 3#	26 ± 3#
Grooming	15 ± 2	8 ± 1*	12 ± 2#	14 ± 2#	13 ± 2#	15 ± 2#
Rotarod Latency (s)	60 ± 5	25 ± 4*	45 ± 5#	55 ± 4#	48 ± 5#	58 ± 4#

Source: Field work, 2025

$p < 0.05$ vs. control; # $p < 0.05$ vs. $AlCl_3$.

3.2. Oxidative stress markers

$AlCl_3$ significantly increased MDA levels and reduced SOD, CAT, and GSH activities, confirming oxidative stress-mediated neurotoxicity (Kumar & Gill, 2014). NLE and tocopherol significantly reversed these

alterations, with superior normalization observed in the high-dose NLE and combination groups.

Table 2. Cerebellar oxidative stress parameters

Group	*Control*	*$AlCl_3$*	*$AlCl_3$ + NLE 200mg*	*$AlCl_3$ + NLE 400mg*	*$AlCl_3$ + Toc*	*$AlCl_3$ + NLE 200 + Toc*
MDA (nmol/mg)	8.2 ± 0.8	18.5 ± 1.2*	12.3 ± 1.0#	9.5 ± 0.9#	11.0 ± 1.0#	8.8 ± 0.8#
SOD (U/mg)	12.5 ± 1.0	5.2 ± 0.5*	8.9 ± 0.8#	11.2 ± 0.9#	9.5 ± 0.7#	12.0 ± 1.0#
CAT (U/mg)	8.5 ± 0.7	3.8 ± 0.4*	6.2 ± 0.5#	7.8 ± 0.6#	6.5 ± 0.5#	8.2 ± 0.7#
GSH (mg/g)	9.2 ± 0.6	4.1 ± 0.3*	6.8 ± 0.5#	8.5 ± 0.6#	7.2 ± 0.5#	9.0 ± 0.6#

Source: Field work, 2025

$p < 0.05$ vs. control; # $p < 0.05$ vs. $AlCl_3$.

3.3. Histological and immunohistochemical findings

Cerebellar sections from $AlCl_3$-treated rats showed marked Purkinje cell degeneration, vacuolation, astrogliosis, and increased caspase-3 expression. These changes were markedly attenuated by NLE and tocopherol, with the combination therapy demonstrating the most pronounced neuroprotection.

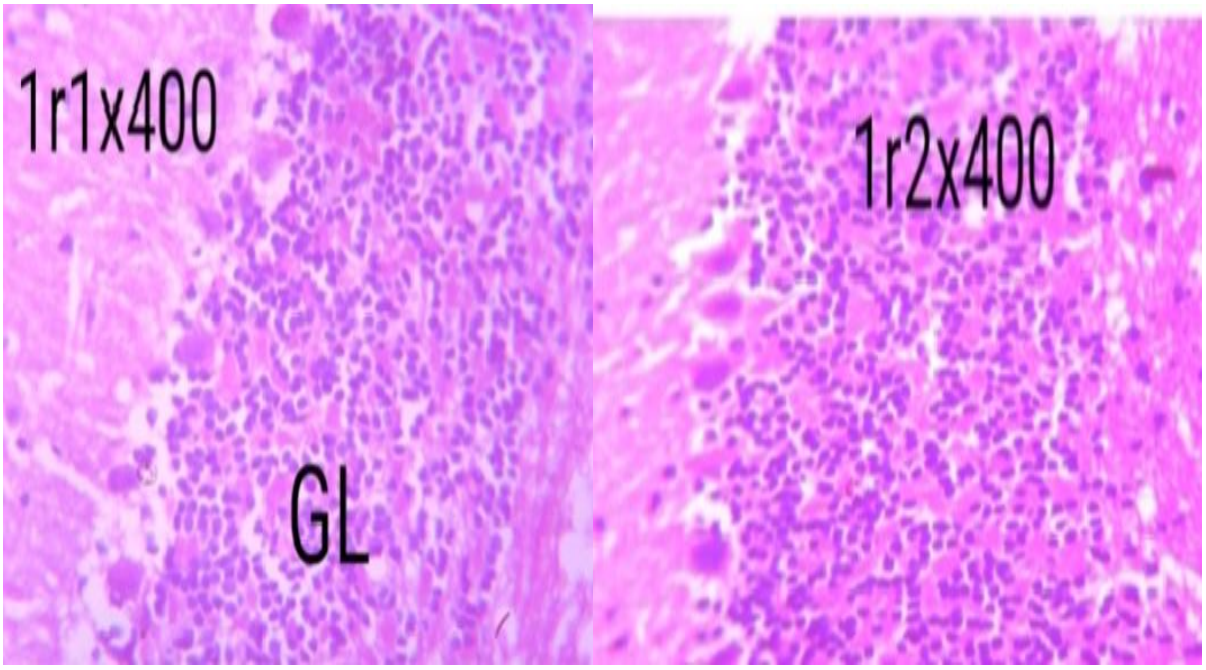

Plate 1. Represents H&E-stained cerebellar sections (×400) which shows normal cerebellum with molecular layer (ML), granular layer (GL) and well outlined prymadial cell within the prymadial cell layer (PLC).)

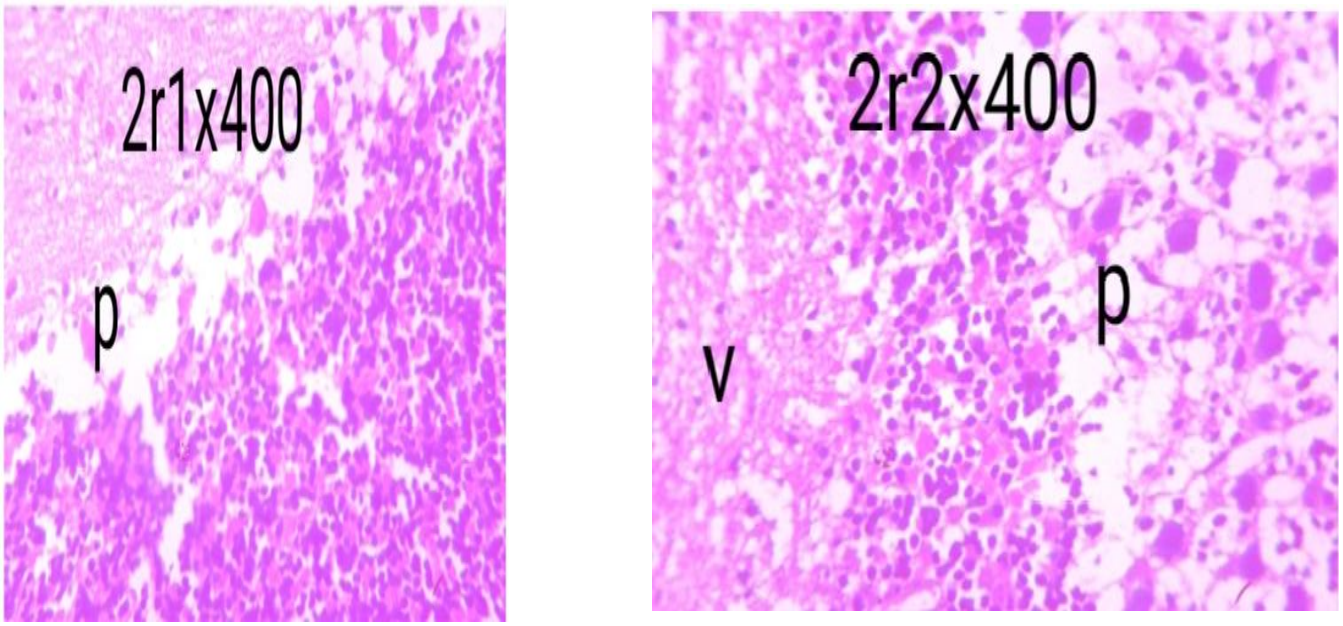

Plate 2: $AlCl_3$ (100mg/kg/day) induced without treatment (X400) shows degeneration and severe vacoulation and severe infilteration of pyknotic prymaidal cell into the granular layer

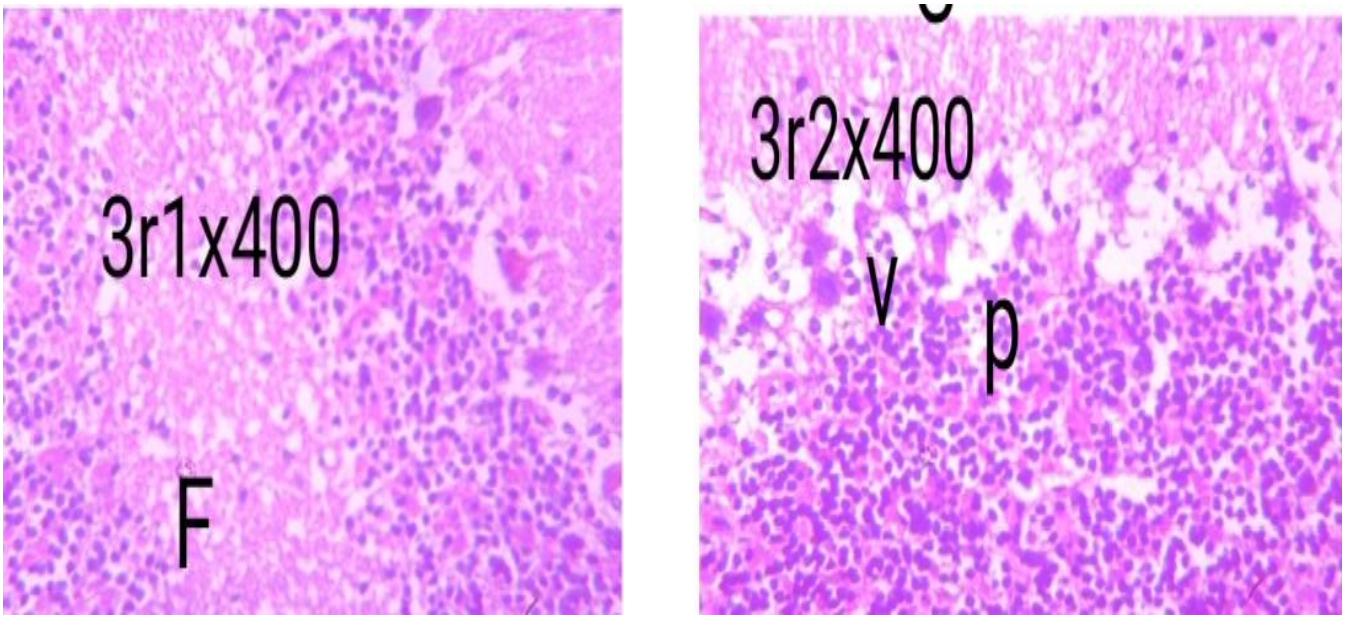

Plate 3: Photomicrograph of group 3 section of cerebellum induced and treated with low dose extract of *Newbouldia laevis (*200mg/kgday*)* (X400/(H/E) which shows mild healing with moderate vacoulation (V), moderate fatty change (FC) and moderate pyknotic (P) prymaidal cell

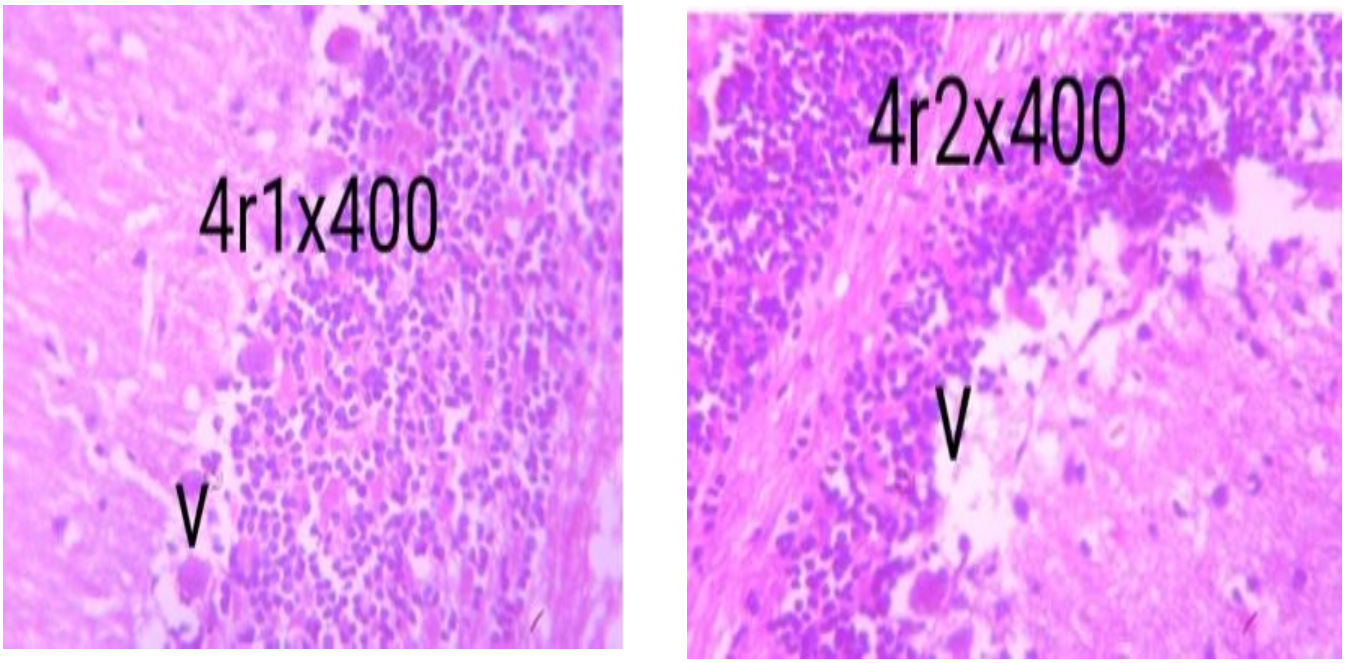

Plate 4: Photomicrograph of group 4 section of cerebellum induced and treated with high dose extract of *Newbouldia laevis* (400mg/kg/day) (X400/(H/E) showing moderate healing with mild vacoulation (V) and mild pyknotic (P) prymaidal cell. The granular cell outline is distinct.

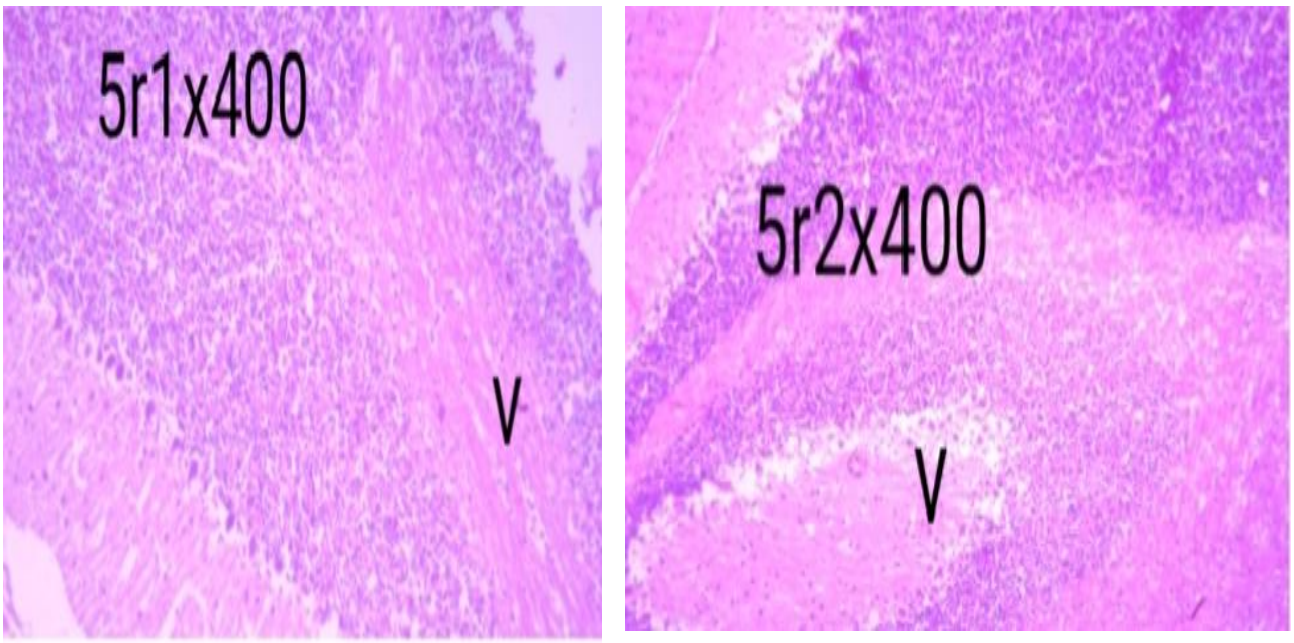

Plate 5: Group 5 treated with $AlCl_3$ + tocopherol (100 mg/kg), X400H/E showing moderate regeneration with mild vacoulation (V) and granular cell,

4. DISCUSSION

The present study demonstrates that $AlCl_3$ induces profound cerebellar oxidative damage, neuronal loss, and motor impairment, corroborating previous findings on aluminium neurotoxicity (Kumar et al., 2009; Ribes et al., 2021). The neuroprotective efficacy of N. laevis leaf extract was comparable or superior to tocopherol, likely due to its rich phytochemical composition and multimodal mechanisms of action.

Flavonoids such as apigenin and quercetin present in N. laevis have been shown to modulate oxidative stress, inhibit apoptosis, and suppress neuroinflammation (Oloyede et al., 2017; Azubuike et al., 2021). The enhanced protection observed in the combination group suggests a synergistic interaction between plant-derived antioxidants and vitamin E, resulting in improved redox homeostasis and neuronal survival.

5. CONCLUSION

Ethanolic leaf extract of Newbouldia laevis confers robust neuroprotection against $AlCl_3$-induced cerebellar cortical damage in Wistar rats. Its efficacy is comparable or superior to tocopherol and is further enhanced when administered in combination. These findings support the therapeutic potential of N. laevis as a natural neuroprotective agent against aluminium-induced neurodegeneration.

REFERENCES

Abd-Elghaffar, S. K. H., Abdel-Latif, M. A. H., Elkomy, A. S., Mohamed, M. A. H., Ahmed, R. & Abdel-Daim M., A. A. (2023). Neuroprotective effect of taxifolin against aluminum chloride-induced dementia and pathological alterations in the brain of rats: Possible involvement of toll-like receptor 4. Life Sciences, 315, 121367.

Adeyemi, O. O., Bassey,I. A., Akinola, S. O., Eyong, E. U. & Ogunlana, T. A. (2022). Antioxidant and Hepatoprotective Effects of Newbouldia laevis.

Akinyemi, A. J., Akinrinade, S. O., Adeyemi, O. O. & Ogunlana, T. A. (2020).

Histomorphological evaluations on the frontal cortex extrapyramidal cell layer following administration of N-Acetyl cysteine. D-NB Info.

Akinyemi, A. J., Akinrinade, S. O., Adeyemi, O. O., & Ogunlana, T. A. (2017). Effect of ginger and turmeric rhizomes on some hemato-biochemical parameters in an experimental model of acetaminophen-overdosed rats. Journal of Ethnopharmacology, 208, 1-8.

Akinyemi, A. J., Akinrinade, S. O., Adeyemi, O. O., Ogunlana, T. A., & Eyong, E. U. (2015). Neuroprotective effect of Allium cepa L. in aluminium chloride induced neurotoxicity. Merck Millipore.

Albrahim, T., Albasher, A., Alrumaihi, F., Almarhoon, A. & Almatroudi, M. M. (2023). Neuroprotective and Cardiometabolic Role of Vitamin E: Alleviating Neuroinflammation and Metabolic Disturbance Induced by $AlCl_3$ in Rat Models. Biomedicines, 11(9), 2453.

Amos, S., Akinmoladun, J. O., Ogunlana, T. A. & Akinyele, I. O. (2005). Sedative effects of the methanolic leaf extract of Newbouldia laevis in mice and rats. Pharmacology Biochemistry and Behavior, 81(3), 584-590.

Azubuike, N. C., Eyong, E. U. & Bassey, A. I. (2021). Anticonvulsant, Muscle Relaxant and In-vitro Antioxidant Activities of Hydroethanol Leaf Extract of Newbouldia laevis Seem. (Bignoniaceae) in Mice. *Journal of Basic Medical Sciences*, 5(1), 1-10.

Azubuike, N. C., Eyong, E. U., Bassey, A. I. & Usman, J. G. (2019). Anxiolytic- and antidepressant-like activities of hydroethanol leaf extract of Newbouldia laevis (P.Beauv.) Seem. (Bignoniaceae) in mice. *Journal of Ethnopharmacology*, 249, 112386.

Bassey, A. I., Eyong, E. U., Adeyemi, O. O., Usman, J. G.& Ogunlana, T. A. (2024). Ninety-Day Toxicological Assessment of Preparation of the Medicinal Plant Newbouldia laevis (P. Beauv.) Seem. (Bignoniaceae) in Rats. Natural Product Communications, 19(5), 1-10.

Chavan, S. S., Patil, P. S., Deshmukh, A. P. & Gawade, R. D. (2023).Tocotrienol-Rich Fraction Ameliorates the Aluminium Chloride-Induced Neurovascular Dysfunction-Associated Vascular Dementia in Rats. Pharmaceuticals, 16(2), 276.

Erbayraktar, Z, Demir, H., Kocak, M., Senol, F. & Arslan, B. (2023). Evaluation of possible neuroprotective effects of virgin coconut oil on aluminum-induced neurotoxicity in an in vitro Alzheimer's disease model. *Journal of Applied Toxicology*, 44(5), 678-689.

Eyong, E. U., Bassey, A. I., Usman, J. G. & Adeyemi, O. O. (2018). Phenolic characterization, antioxidant activities, and inhibitory effects of Physalis angulata and Newbouldia laevis on enzymes linked to erectile dysfunction. *International Journal of Food Properties*, 21(1), 645-654.

Eyong, E. U., Bassey, A. I., Usman, J. G. & Akinrinade, S. O. (2013). Trado-Medical Uses, Chemical Constituents and Biological Activities of Newbouldia laevis (Bignoniaceae). Pharmaceutical Sciences, 19(2), 51-60.

Godam, E. T., Adeyemi, O. O., Usman, J. G., Ogunlana, T. A. & Bassey, A. I. (2024). Neuroprotective Effects of Phoenix dactylifera L. and Melatonin on Aluminum Chloride-induced Neurotoxicity in the Prefrontal Cortex of Male Wistar Rats. *Journal of Anatomical Sciences*, 21(2), 189-200.

Kumar, V., Gill, K. D., Sharma, P. K. & Singh, S. (2009). Aluminium-induced oxidative stress in rat brain: Response to combined administration of citric acid and HEDTA. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 149(4), 503-514.

Majumdar, S., Kumar, R., Singh, A. K. & Singh, M. (2014). Coenzyme Q10 Abrogated the 28 Days Aluminium Chloride Induced Oxidative Changes in Rat Cerebral Cortex. PubMed Central.

Maya, S., Kumar, P., Singh, R. & Sharma, V. (2016). Evaluation of neuroprotective effects of wedelolactone and gallic acid on aluminium-induced neurodegeneration: Relevance to sporadic amyotrophic lateral sclerosis. *European Journal of Pharmacology*, 771, 113-121.

Nedzvetsky, V. S., Vlasov, A. A., Babenkova, E. P. & Zheltova, I. A. (2006). Effects of vitamin E

against aluminum neurotoxicity in rats. Biochemistry (Moscow), 71(3), 239-244.

Ogundipe, O. O., Bassey, A. I., Usman, J. G. & Eyong, E. U. (2024). The Neuroprotective and Therapeutic Effects of Medicinal Plants and Natural Products against Aluminium Chloride-Induced Alzheimer's Disease. Science Biology.

Ogunlade, B., Bassey, A. I., Ogunlana, T. A. & Usman, J. G. (2022). Neuroprotective (Antioxidant, Antiamyloidogenic, and Antiexcitatory) Effects of Trévo against Cadmium Chloride Neurotoxicity in Adult Male Wistar Rats. OBM Neurobiology.

Ogunlana, O. O., Bassey, A. I., Eyong, E. U. & Usman, J. G. (2023). Pharmacognostic profiles, evaluation of analgesic, anti-inflammatory and anticonvulsant activities of Newbouldia laevis (P. Beauv.) Seem. ex Bureau leaf and root extracts in Wistar rats. *Journal of Ethnopharmacology*, 300, 115736.

Ogunlana, O. O., Bassey, A. I., Eyong, E. U. & Usman, J. G. (2024). Ethanol Leaf Extract of Newbouldia laevis Attenuates Stress-Induced Prefrontal Cortical Damage by Reducing Lipid Peroxidation and Corticosterone Level in Adult Wistar Rats. *Anatomy Journal of Africa*, 13(1), 45-56.

Ogunlana, O. O., Bassey, A. I., Eyong, E. U. & Usman, J. G. (2025). Effect of Newbouldia Leavis Aqueous Extract on Cardiac Troponin, Creatinine Kinase, Myoglobin and Lactate Dehydrogenase in Albino Rats Treated with Diclofenac. ResearchGate.

Ojo, O. A., Eyong, E. U., Usman, J. G. & Bassey, A. I. (2020). Antioxidant and Neuroprotective Activities of the Mesocarp of Raphia hookeri Fruit against Aluminum Chloride-Induced Neurotoxicity in Rats. Scirp.org.

Oliveira, C. S., Silva, M. F., Santos, R. A. & Gomes, P. H. (2018). Neuroprotective effect and antioxidant activity of Passiflora edulis fruit flavonoid fraction, aqueous extract, and juice in aluminum chloride-induced Alzheimer's disease rats. Nutrire, 43(1), 1-12.

Oloyede, G. K., Eyong, E. U. & Bassey, A. I. (2017). Apigenin: A methanol fraction component of Newbouldia laevis leaf, as a potential antidiabetic agent. *The Journal of Phytopharmacology*, 6(1), 38-44.

Oloyede, G. K., Eyong, E. U., Bassey, A. I. & Usman, J. G. (2021). Inhibition of oxidative stress and gastric emptying as additional mechanisms of antidiabetic activity of Newbouldia laevis. Phytomedicine Plus, 1(1), 100005.

Oluwafemi, A. J., Eyong, E. U., Bassey, A. I. & Usman, J. G. (2024). Effects of Newbouldia laevis (P. Beauv) Aqueous Leaf Extract on Liver Function and Histology in Mercury Chloride-Induced Wistar Rats. *British Nigerian Academy of Sciences Journal*, 1(1), 45-55.

Patel, P. K., Sharma, V. K., Singh, A. K. & Kumar, R. (2022). Neuroprotective Effect of Ethanolic Extract of Galinsoga parviflora Plant against Aluminium Chloride (AlCl3) induced Neurotoxicity in Rats. Global Research Online, 76(2), 150-160.

Rajendran, S., Kumar, P., Sharma, A.& Singh, V. (2023). Effect of vitamin-E on aluminium chloride induced toxicity in cerebral cortex of wistar albino rats: A histological and biochemical study. *International Journal of Anatomy and Research*, 11(2), 8567-8574.

Ribes, D., Sabaté, J., Guimerà, M. & Alomar, A. (2021). Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. Advances in Neurotoxicology, 5, 1-34.

Sharma, S., Kumar, V., Singh, A. K. & Gupta, R. (2024). Vitamin E protects against neurotoxicity in a rat Alzheimer's model. World Journal of Pharmaceutical Research, 13(15), 1-10.

Singhal, N. K., Srivastava, A. K. & Mishra, R. K. (2015). Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: behavioral and biochemical alterations in rats. Biometals, 28(2), 379-390.

Tagne, R. S., Kamga, J. M. & Tchoumbougnang, P. N. (2014). Neuroprotective effect of Baillonella toxisperma Pierre on the oxidative stress status in an experimental animal model of Alzheimer's disease. RAJ Pub.

Usman, J. G., Eyong, E. U., Bassey, A. I. & Adeyemi, O. O. (2015). Phytochemical and Biological Effects of Newbouldia laevis: A Review. Planta Escientia, 2(1), 10-20.

Usoro, E. J., Bassey, A. I., Usman, J. G. & Eyong, E. U. (2023). Phytochemical constituents and haematological effect of hydro-ethanolic extract of Newbouldia laevis leaves on glyphosate induced. *OAJI Journal*, 10(4), 100-110.